STRUCTURE AND FUNCTION OF THE TIBIAL GLAND OF THE AUSTRALIAN FROG *LIMNODYNASTES DUMERILI* PETERS

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Summary

G. A. CROOK & M. J. TYLER (1981) Structure and function of the tibial gland of the Australian frog *Limnodynastes dumerili* Peters. *Trans. R. Soc. S. Aust.* 105(2), 49-52, 12 June, 1981.

Limnodynastes dumerili has a large, oval, dermal gland on the tibia; it is perforated with ducts at a density of 6/mm². In horizontal section the gland appears as a series of large horizontal chambers filled with secretions. The ontogeny of glands in tadpoles is described.

When the gland secretion is expressed manually to the surface, rats avoid the frog. In the absence of expression the frog is eaten but the glands are left untouched. Predators of the frog and closely related species with similar glands are listed.

Introduction

In the Anura there are diverse kinds of discrete dermal glands. Examples are the parotoid glands of many species of *Bufo* (Blair 1972); the dorsal lipid glands of South American phyllomedusine hylids (Blaylock *et al.* 1976); the supralabial glands of *Rana* (Smith 1954) and the tibial glands of some species of *Bufo* (Blair 1972) and some Australian frogs of the genus *Limnodynastes* (Martin 1972; Tyler 1976).

Much attention has been devoted to parotoid glands and their secretions (Low 1972); the structure and possible functions of the tibial glands have not been examined. Here we describe the gross structure and ontogeny of these structures in the southern Australian species *Limnodynastes dumerili*, and investigate their possible role.

Limnodynastes dumerili is a robust species measuring up to 75 mm snout to vent length when fully grown. It is widely distributed in southeastern Australia and is a member of the *L. dorsalis* group of species, whose representatives extend over the eastern and southwestern portions of the continent (Martin 1972). Illustrations of the frog appear in Barker & Grigg (1977), Tyler (1977, 1978) and Cogger (1979).

Material and methods

The specimens used in this study were collected at various localities within an 80 km radius of Adelaide and were maintained for varying periods in vivaria in the University of Adelaide.

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Glands were obtained from freshly decerebrated and spinalised animals. They were dissected free from the tibia and for histological preparations were fixed in 10% buffered formalin. Sections were cut at 7 μm and stained with haematoxylin and eosin. For studies where glands were exposed to potential predators, minimal time elapsed between the death of the donor and such exposure. In six trials, groups of four rats were fasted for 48 hours and the entire dead frog offered, the entire frog minus the glands, or gland secretions wiped upon the surface of rat pellets or fresh meat.

Studies of ontogeny were based on tadpoles collected at stages 25-32 of Limbaugh & Volpe (1957) and reared on a diet of boiled lettuce leaves. Samples were taken from this stock at intervals determined by hindlimb development.

Gross and histological structure

The tibial glands of *Limnodynastes dumerili* are single, raised, ovoid, dermal structures on the dorsal surface of each tibia and occupying more than 50% of the dorsal aspect of that limb segment (Fig. 1). Dimensions of the gland in a 75 mm frog are 15 x 11 mm. In a series of nine specimens the length of the gland was 51-69% of the length of the tibia. The gland is entirely dermal and does not adhere to underlying muscles. It is palpable and resilient. The dorsal surface of the gland is perforated by the apertures of numerous ducts at a frequency of approximately 6/mm². In some frogs the gland is pigmented with a metallic sheen contrasting with the remainder of the dorsal skin, however in the majority there was no such colour differentiation.

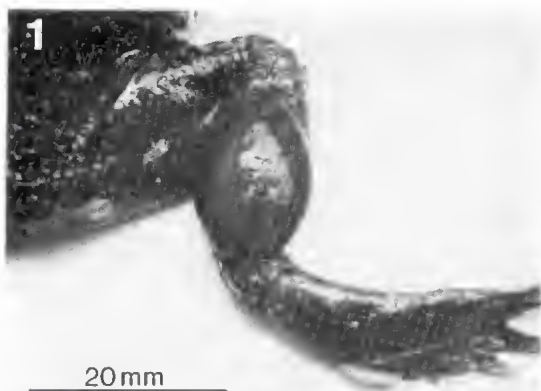In transverse section (Fig. 3) the gland has a regular, columnar arrangement of cavities

Fig. 1. Dorsal aspect of right hindleg of *Limno-dynastes dumerili* showing large, protuberant tibial gland.

filled with vast quantities of secretion, and communicating to the exterior via short, narrow ducts. The stratum corneum is of moderate thickness, and mucus glands are comparable in size with those of other frog species. In horizontal section (Fig. 2) the regular shape and repetitive form of the secretory cavities is revealed. In the illustration the secretory contents have contracted from the very narrow matrix of connective tissue. We attribute this to the dehydration process during histological preparation. At higher magnifications a few small, circular vesicles could be detected within the secretory material.
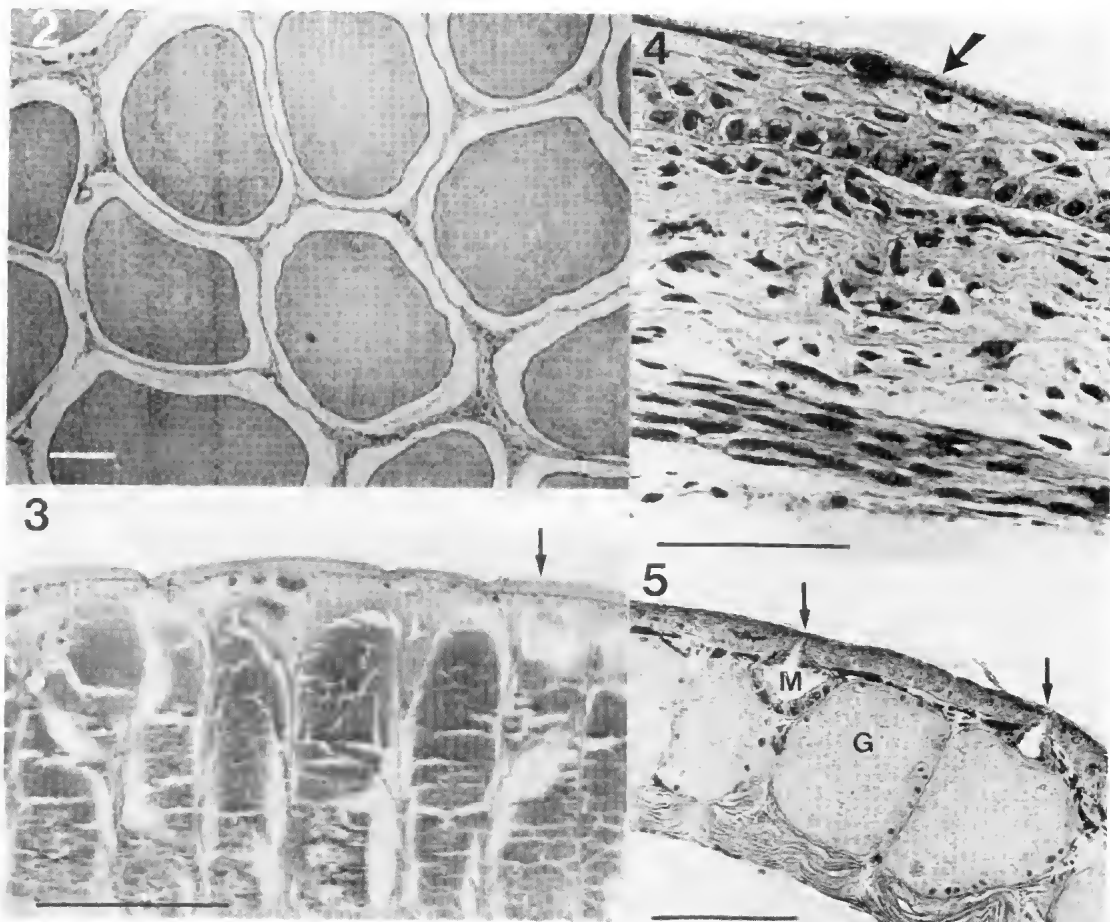

Fig. 2. Horizontal section of tibial gland. Dense areas are the internal secretions of the gland. These secretions have contracted slightly. Scale bar = 100 μm. Fig. 3. Transverse section of portion of tibial gland. Note columnar arrangement of the internal matrix, and duct (arrowed). The smear of secretions in the gland is fractured as a result of histological preparation. Scale bar = 1 mm. Fig. 4. Transverse section of skin of tibial region of tadpole at stage 39 of Limbaugh & Volpe (1957) Note occurrence of cell nuclei in most superficial portion of stratum corneum (arrowed). Scale bar = 50 μm. Fig. 5. Transverse section of skin of tibial region of tadpole at stage 43 of Limbaugh & Volpe (1957). Granular glands (G) and mucus glands (M) are well developed and communicate with the surface via open ducts (arrowed). Scale bar = 100 μm.

The gland is innervated by a braneh of the seiatic nerve, and there is a network of blood vessels upon the ventral surface of the gland.

The tibial glands first beeome evident in the tadpole at stage 42 and become more prominent until, at stage 46, they assume the oval form characteristie of those of the adult. At stage 39, the general adult strueture of the skin is recognizable (Fig. 4), but the second layer of the stratum corneum contains cells with nuclei whereas in the adult no nuclei oceur there. The stratum spongiosum is very thin and non-glandular. Glands are represented in the epidermis by aggregations of cells.

At stage 40 the ultimate layering of skin is distinguishable. Glands are present in the stratum spongiosum and some have formed lumina. The evidence suggests that the glands develop downwards into the stratum spongiosum, and continue to enlarge there. By stage 41 the lumina of the granular glands are fully formed and secretion by the now comparatively thin inner layer of epithelial cells has begun. The secretion is in the form of spherieal globules. Ducts conneet the glands to the surface. The epidermis has changed and assumed the adult form with enucleate stratum eorneum cell layers. Aggregations of epidermal cells represent incipient mucus glands. By stage 43 the mucus glands have developed lumina containing mucus. The granular glands have expanded to occupy nearly the entire stratum spongiosum (Fig. 5).

At stage 44 granular glands in the tibial gland are in various developmental states. The glands at the centre have expanded to occupy the entire stratum spongiosum and are very tightly packed together. However, the peripheral ones are small and less dense. By stage 45 the mucus glands are well developed and the granular glands oeeupy the entire stratum spongiosum. At stage 46 the granular glands have increased further in area and the stratum spongiosum has expanded to accommodate them. The glands are tightly packed and thus comparable with the form of the adult tibial gland.

Function of tibial glands

Beeause the tibial gland appears during late larval life, it is clearly of funetional significance only to the adult. The potential range of functions is extensive, but protection from predators appeared the most fruitful direction of investigation. Examination of the literature established that *L. dumerili* and its close relatives sharing tibial glands are normally eaten by a wide variety of vertebrates (Table 1).

We fed freshly killed frogs to several vertebrate predators: *Chelodina longicollis*, Freshwater Tortoise, ate tadpoles and juvenile frogs with no adverse effects: *Notechis scutatus*, Tiger Snake (no effect upon the snake); *Anas platyrhynchos*, Mallard (juveniles up to 40 mm in length eaten readily) and also to *Rattus norvegicus*, rat. Rats ate every portion

TABLE 1. *Predation upon frogs of the* **Limnodynastes dorsalis** species group

Species of frog	Predator	Details	Source
*L. dorsalis**	*Vulpes vulpes* (Red Fox)	In 1% of stomachs investigated, 1958 ⎫ In 7.7% of stomachs investigated, 1959 ⎬	McIntosh 1963
L. dumerili†	*V. vulpes* (Red Fox)	1 in stomach	J. D. Croft (pers. comm.)
L. dumerili‡	*Notechis scutatus* (Tiger Snake) ⎫ *Pseudechis porphyriacus* (Black Snake) ⎬	Fed to captive specimens	E. Worrell (pers. comm.)
L. dumerili	*Litoria raniformis* (frog)	Observed swallowing *L. dumerili* on two occasions. Predator unaffected	M. J. Tyler (unpublished)
L. dumerili	Owls	Bones of numerous frogs found in regurgitated owl pellets	M. J. Tyler (unpublished)

* Frog species identified as "*L. dorsalis*" were reclassified as a complex of species by Martin (1972).
† Identity uncertain: captured in areas of sympatry between *L. dumerili* and *L. terraereginae*.
‡ Identity uncertain: locality data unknown.

of the frog except the tibial glands and a small strip of skin joining the glands together. The experiment was performed six times with the same results: namely that rats fasted for 48 hours would eat the entire *L. dumerili* with the sole exception of the tibial glands. However in the experiments in which exudate from the glands was expressed onto the outer surface of the skin, the fasted rats would not eat any part of the frog. Having appeared to taste the gland's contents they exhibited great distress, running around the cage, and making wiping motions with their paws. However, when the tibial glands were removed from the frog, the rats ate the hind limbs with the rest of the body.

When food objects were coated with the secretion and offered to fasted rats, the items were rejected after initial investigation (usually by licking, and this activity was followed by extensive cleaning of the mouths and paws).

Discussion

We have not performed pharmacological or biochemical investigations to determine the identity of the glandular secretion, but we note that in their survey of pharmacologically active substances from the skin of Australian frogs, Roseghini *et al.* (1976) reported that in the *L. dorsalis* group a variety of substances widely distributed in other species was absent, with the exception of 5-Hydroxytryptamine (5-7 μg/g skin). Although we are unable to identify the substance involved, our observations indicate that the secretions of the tibial glands afford the animal a degree of potential protection from at least some of the animals likely to predate upon it if the secretions are released from the glands. However the efficacy of the mechanism is uncertain. The secretions can be expressed by applying lateral pressure to the glands, but we have failed to observe release under other forms of stress.

Acknowledgments

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